Nudging Through a Nucleosome

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Herschel is an enormously versatile space telescope. It will study the physics and molecular chemistry of almost all types of cool celestial objects, from our own neighborhood to the edge of the Universe. The nearest objects it will study are within our own solar system, such as comets. These mountain-sized chunks of ice and rock are the leftovers from the formation of the planets, more than 4 billion years ago. They are the best fossils from the early solar system and can tell us what raw ingredients became the planets, including Earth.

It will also look into the dense clouds of matter that enclose stars in the process of formation. The ISO mission unveiled more than a dozen of these regions, but Herschel will find many more and will be able to look inside them to see the star-forming process happening. It will also look at the rings of debris that accumulate around forming stars, where it is believed that planets are completing the process of formation.

In addition, the telescope will look at young galaxies in the distant universe. Today, galaxies are giant collections of hundreds of billions of stars. The first objects that formed in the early universe were much smaller and then grew by merging together in dramatic collisions. These collisions triggered enormous bouts of star formation. The first census of star-forming galaxies will be made throughout the universe at the epoch of peak star formation, allowing the star-formation history and evolution of galaxies in the universe to be charted. The youngest stars in our Galaxy will be revealed, as will the vast reservoirs of gas and dust that constitute half the normal matter.

When the Hubble Space Telescope took its historic images of the distant universe in the 1990s, it saw a new population of distant, irregularly shaped galaxies. The James Clerk Maxwell Telescope on Hawaii also looked at these regions at a wavelength of 850 μm. It too saw distant galaxies, but different ones from Hubble. Herschel operates at wavelengths that bridge the gap between these two instruments and will show us the relationship between these apparently different young galaxy populations.

If Herschel were placed in orbit around Earth, heat from our planet would interfere with its instruments, reducing their sensitivity. Instead, Herschel will orbit a point in space about 1.5 million kilometers from Earth. Called the second Lagrangian point (L2) of the Sun-Earth system, it is a local gravitationally stable point providing an excellent place for Herschel to shelter from the heat being emitted by Earth, with a good view of the sky. A sun shield will protect the telescope from the Sun's radiation, which Herschel needs to be bathed in to power its solar arrays. Three years of routine science operations are planned, at the end of which there will be the option to extend the mission if the spacecraft is in good health and still has some of its 2400 liters of helium coolant left.

So far, about 60% of the observing time on Herschel has been allocated to 42 large Key Programs (*4*–*6*). Half are Guaranteed Time programs led by the instrument teams and half are Open Time programs competed for by the astronomical community. The remaining 40% of the observing time will be allocated by competition about a year into the mission. The 42 programs allocated so far cover an exciting range of science. In the solar system, the chemistry of water and trans-Neptunic objects will be studied. There will be studies of debris disks around stars, protoplanetary systems, and analogies of the solar system's distant Kuiper belt. Programs will study interstellar dust and molecules as well as star-forming regions in our Galaxy, and there will be a survey of the whole Galactic plane. There will be detailed studies of nearby galaxies, including the Magellanic Clouds, the Virgo cluster, and gravitational lens systems. There will be several large-scale cosmological surveys, of which the largest is the Hermes multilayered survey.

These two great missions, Herschel and Planck, were first proposed 13 years ago, in 1996, and had been studied for several years before that. Although their technical complexity has meant that the launch was later than originally planned, the expected insight they will provide into the cold and dusty regions from which planets, stars, and galaxies form, and into the early universe, means it has been well worth waiting for.

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BIOCHEMISTRY

Nudging Through a Nucleosome

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Single-molecule data suggest that RNA polymerase II moves a small step forward only when its DNA template briefly unwraps from the histone core.

Medieval monks feverishly transcribing Latin into Olde English would identify with the struggles that the eukaryotic RNA polymerase II complex must overcome in order to write DNA in the language of RNA. While theirs was a conceptual barrier, that of RNA polymerase II is quite physical and embodied by nucleosomes. Much like string wrapped around a spool, nucleosomes consist of a cylindrical protein core and DNA wrapped tightly around it. On page 626 of this issue, Hodges *et al.* (*1*) report single-molecule measurements that help to elucidate how the nucleosomal DNA is transcribed.

One of the nucleosome's functions is to compact genomes in eukaryotic nuclei. This compaction acts as a barrier to transcription (the process by which an RNA polymerase II enzyme converts a DNA sequence into mRNA). During transcription, RNA polymerase moves along double-stranded DNA, locally separating it into two single strands. One DNA strand is passed through the enzyme's active site, where its base sequence is read and the appropriate ribonucleotides are added to the end of a growing RNA strand. To transcribe nucleosomal DNA, the interactions between the nucleosomal core and the DNA backbone must be disrupted, because these interactions would otherwise cause the polymerase to slow down or stop transcribing.

To probe the dynamics of a single polymerase II molecule while it transcribes nucleosome-bound DNA, Hodges *et al.* used optical tweezers. In an optical tweezing experiment, a laser beam is tightly focused on a particle, typically a transparent polystyrene bead, suspended in solution. The change in the momentum of the photons refracted by the bead-solution interface pushes the bead to the center of the focused beam and traps it there. Movement of the bead away from the beam center by any external force is measured by monitoring the position of the bead. If a pair of laser beams holds two beads con-

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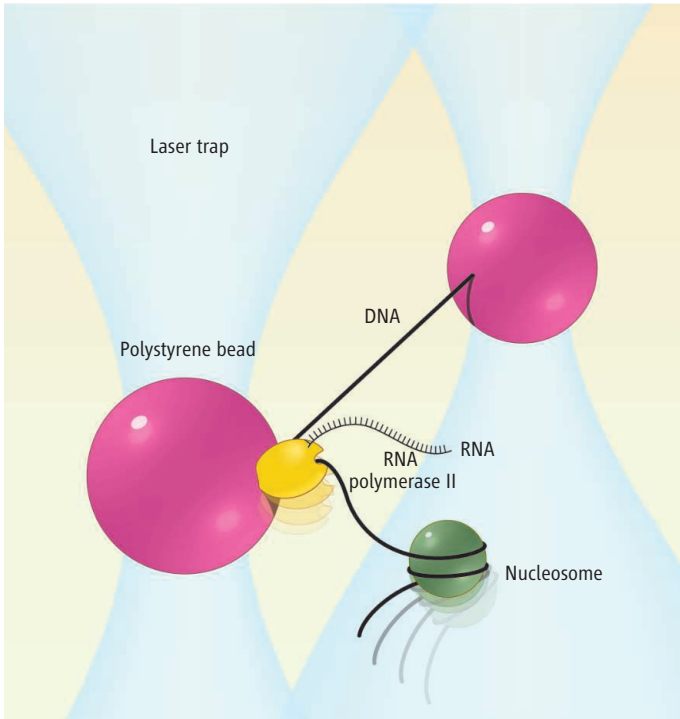
nected to one another through a single DNA-protein tether, the entire system can be suspended in solution and separated from laboratory noise and drift, improving the resolution of force and distance measurements.

This type of setup has been used to show that the step size of a transcribing polymerase equals the separation between DNA base pairs and that during transcription, the polymerase moves forward via a Brownian ratchet mechanism: The molecule diffuses along the DNA template until it is rectified through binding of the next nucleotide called for by the DNA sequence (*2*). However, this mechanism is frequently interrupted by pauses that depend on the DNA sequence (*2*, *3*). These pauses have been attributed to a diffusive backtracking mechanism (*4*).

Backtracking of a polymerase occurs when the nascent RNA transcript becomes fed backward through the polymerase molecule, thereby occluding the enzyme's active site and prohibiting further nucleotide addition; with its active site blocked, the polymerase diffuses back-and-forth in one dimension along the DNA-RNA hybrid until the Brownian movement realigns the RNA 3′-end with the active site, allowing ribonucleotide addition and transcription to continue. Previous bulk biochemical work has shown that both sequence-dependent pausing and backtracking are exacerbated in the presence of a nucleosome (*3*, *5*), but the ensemble averaging in these studies occluded many of the mechanistic and kinetic details.

In their dual-trap assay, Hodges *et al*. first loaded a polymerase II molecule onto a DNA sequence that contained both intrinsic pause sites and a nucleosome-positioning sequence (either with or without a downstream nucleosome). At this point, the enzyme is stalled but poised to transcribe. They attached this stalled enzyme to one polystyrene bead and the DNA upstream of the enzyme to a second bead, thereby creating a DNA-based tether that was pulled taut by tuning the distance between the two beads (see the figure). Finally, they added a ribonucleotide-containing solution, thereby triggering polymerase transcription. The resulting lengthening of the upstream DNA could be measured by an increase of the distance between the two beads.



Dual trap. Hodges *et al*. attached a single RNA polymerase II molecule (preloaded onto 3 kb of double-stranded DNA) to an optically trapped bead (front). They affixed the upstream end of the DNA to another bead in a second optical trap (back). The movement of the polymerase II on the DNA can be measured by a lengthening of the tether length between the beads, allowing the interaction between the polymerase II and a downstream nucleosome to be studied.

This setup enabled Hodges *et al*. to quantify the effects of a nucleosome that lies in the path of a polymerase. They found that when transcribing through a nucleosome, the local probability for polymerase II to pause along the template tripled and that the region of highest pause density lies in the first half of the nucleosome-bound DNA. Further, the median pause duration doubled and the transcriptional velocity fell by 40%.

By comparing pause-duration distributions from many repeats of the experiment to a mathematical model describing the RNA polymerase II as a one-dimensional random stepper in the absence of any barriers (*6*), Hodges *et al*. were able to understand the role of polymerase II diffusion in bypassing a nucleosome. The authors incorporated the nucleosome barrier into the stepper model by assuming that the nucleosome fluctuates rapidly between two states: one in which the DNA is partially unwrapped in front of the polymerase, and another in which it is completely wrapped around the nucleosome core. Because the stepper (polymerase II) can move forward only when the nucleosome is partially unwrapped, the authors scaled the probability of moving forward by the fraction of time the nucleosome is in the partially unwrapped state. This assumption successfully predicts transcriptional and pausing kinetics in very good agreement with those measured experimentally. They conclude that during both backtracking and forward transcription through a nucleosome, the polymerase advances by taking advantage of fluctuations that partially unwrap nucleosomal DNA. In this manner, polymerase II acts to rectify brief nucleosomal openings as it ratchets through nucleosomal DNA.

The observation that polymerase II behaves as a diffusion-based Brownian ratchet when it pauses and backtracks is in agreement with previous single-molecule studies demonstrating this ratchet mechanism during normal transcription (*2*). It is also consistent with previous work suggesting that as the nucleosome rewraps, it can induce backtracking and push the polymerase backward through the reformation of contacts between DNA and the nucleosomal core (*3*).

These observations could have an impact on the field of transcriptional regulation and epigenetics. When nucleosomes in human embryonic stem cells and in *Drosophila melanogaster* are posttranslationally modified in a certain pattern, polymerase II will begin to transcribe a gene but soon becomes arrested. With the addition of a single nucleosomal modification, the gene is quickly transcribed in its entirety (*7*–*9*). Such nucleosomal modifications, or factors recognizing them, could affect polymerase II's nucleosome passage simply by altering the fraction of time a nucleosome fluctuates into an unwrapped state. It remains to be seen whether nucleosome modifications play such a mechanistic role, but Hodges *et al*. show that whether transcribing words or DNA, you've just got to ratchet through one bit at a time.

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